ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1982, p. 323-329
0066-4804/82/080323-07$02.00/0

Vol. 22, No. 2

Transposon-Mediated Multiple Antibiotic Resistance in *Acinetobacter* Strains

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Received 19 April 1982/Accepted 12 May 1982

Acinetobacter calcoaceticus subsp. *anitratus*, which is unusually resistant to multiple antibiotics, was the cause of an epidemic of respiratory tract infections in patients in an intensive care unit. A representative isolate of the epidemic strain was found to contain the aminoglycoside-modifying enzymes 3-*N*-acetyltransferase, 3'-phosphotransferase, and 3"-adenylyltransferase, which confer resistance to gentamicin, kanamycin, and streptomycin, respectively. In addition, the strain produced a cephalosporinase and was resistant to penicillins due to the production of a TEM-2 β-lactamase. The bacterial isolate also exhibited resistance to chloramphenicol, tetracycline, and sulfonamides. The resistant phenotype of this strain was similar to resistance patterns frequently observed in endemic hospital flora, suggesting that the transfer of an R plasmid into *Acinetobacter* sp. may have occurred. However, antibiotic resistance could not be transferred to any recipient by various mating procedures. After plasmid RP4 was transferred into an ampicillin- and kanamycin-susceptible derivative of the epidemic strain, mobilization of resistance to chloramphenicol, gentamicin, streptomycin, sulfonamides, and possibly tetracycline could be achieved. This mobilization was due to the transposition of a 16-megadalton DNA sequence from the *Acinetobacter* chromosome into plasmid RP4. Insertion of the transposable sequence occurred near the *Pst*I and *Sma*I sites around position 22.5 on the physical map of plasmid RP4. We suggest that a plasmid resistant to multiple antibiotics was transferred from the hospital flora into *Acinetobacter* sp. but could not be maintained stably in this host. Instead, a multiply resistant DNA sequence was transposed and stably integrated into the *Acinetobacter* chromosome. The occurrence of such multiply resistant transposons on conjugative plasmids contributes greatly to the genetic variability of bacteria and may sometimes have serious epidemiological and therapeutic consequences.

Acinetobacter calcoaceticus is a ubiquitous organism which is found mainly in water, soil, and food and in lower animals as part of their normal flora. Only recently has this organism been found to be a possible cause of nosocomial infections in compromised hosts (7, 19). At the Institute of Medical Microbiology, Zurich, Switzerland, we isolate between 250 and 350 strains of *Acinetobacter* sp. per year from specimens obtained from hospitalized patients. These strains constitute about 1 to 2% of all members of the *Enterobacteriaceae* family and oxidative-fermentative bacteria cultured each year in our laboratories. At the end of 1977, however, we observed a sudden fivefold increase in the number of isolations of *Acinetobacter* sp. This increase was due to an epidemic occurring in one of the intensive care units of the University of Zurich hospital. All *Acinetobacter* strains isolated from patients in that unit were *A. calcoaceticus* subsp. *anitratus* and were resistant to multiple antimicrobial agents. Since the resistant phenotype was identical to a plasmid-specified resistance pattern frequently observed in the gram-negative hospital flora, we suspected that transfer of an endemic plasmid from the hospital flora to *Acinetobacter* sp. might have occurred. However, preliminary experiments did not give evidence of a resistance plasmid in the epidemic strain. Studies, therefore, were performed to obtain information about the biochemical mechanism and the genetic basis of resistance in this strain. This paper summarizes the results of our investigation.

MATERIALS AND METHODS

Bacterial strains. *A. calcoaceticus* HK302 was isolated from the respiratory tract of a patient in the intensive care unit. As recipients for transfer of resistance from HK302, various *Escherichia coli*, *Pseudomonas aeruginosa*, and *A. calcoaceticus* wild-type strains were used. *E. coli* HB101 (*recA*) was used as the recipient for mobilization experiments involving plasmid RP4. *E. coli* K-12 (J53) was the host of plasmid RP4, which carries resistance to ampicillin,

kanamycin, and tetracycline (12). The source of reference plasmids used for molecular-weight determinations in agarose gel electrophoresis was *E. coli* V517 (26).

Susceptibility testing. Susceptibility testing was performed either by the single-disk diffusion method (standardized according to the U.S. standard [1]) or by a broth dilution procedure with Mueller-Hinton broth (Oxoid Ltd., Basingstoke, England) as the nutrient medium (18).

Enzyme isolation and assays. Aminocyclitol-modifying enzymes were isolated by the osmotic shock procedure (30). Enzymatic activity in crude extracts was examined by the phosphocellulose-paper-binding assay (32). Details of the procedures used are given elsewhere (17). β-Lactamases were obtained either by osmotic shock (30) or by treatment of cells with ultrasound (23).

Substrate profiles were determined by the iodometric method (33). The profiles were calculated against an arbitrary value of 100 for the rate of hydrolysis of benzylpenicillin.

Isoelectric focusing of crude enzymatic extracts was performed with an LKB 8100 electrofocusing column (LKB Produkter AG, Bromma, Sweden) by using the procedure described by the manufacturer. Fractions (4 ml each) were adjusted to pH 5.9 and assayed iodometrically.

Enzymatic degradation of chloramphenicol was measured in overnight broth cultures containing 50 mg of the drug per liter. Bacteria were removed by centrifugation, and the supernatant was heated at 90°C for 5 min. Samples were assayed in a bioassay (2), with *Sarcina lutea* ATCC 9341 as the test organism.

Conjugation experiments. The transfer of antibiotic resistance markers from *A. calcoaceticus* to appropriate recipients was performed either on brain heart infusion agar according to the method of Towner and Vivian (37) or in brain heart infusion broth as described by Olson and Shipley (31). Transconjugants were selected as described below. The counterselecting agent was either nalidixic acid or streptomycin at a concentration of 100 or 2,000 mg per liter of agar, respectively.

Curing. Elimination experiments were performed with ethidium bromide in brain heart infusion cultures (5). Storage of brain heart infusion cultures at 35°C for several weeks was also used as a plasmid-curing procedure.

Isolation and characterization of plasmid DNA. A 1-ml portion of an overnight culture was inoculated into 100 ml of a semisynthetic medium (0.7% Na_2HPO_4, 0.05% NaCl, 0.1% NH_4Cl, 0.3% KH_2PO_4, 0.02% $MgSO_4$, 0.002% $CaCl_2$, 0.4% glucose, 0.4% Casamino Acids [Difco Laboratories, Detroit, Mich.], 0.025% thiamine HCl [pH 7.2]) and incubated at 37°C. After 90 min of incubation, 250 mg of deoxyadenosine per liter was added (6), and 5 min later, either 5 μCi of [*methyl*-^{3}H]thymidine (specific activity, 100 Ci/mmol) or 0.5 μCi of [2-^{14}C]thymidine (specific activity, 50 mCi/mmol) per ml was added. Incubation was continued until the end of logarithmic growth. Cells were lysed, and plasmid DNA was isolated by isopycnic centrifugation according to the methods of Clewell and Helinski (10) and Lovett and Helinski (25). To obtain plasmid DNA in large amounts, the lysis and purification procedures were identical, except that cultures were grown in 1.5 liters of L-medium (1% tryptone [Difco], 0.5% yeast extract [Difco], 0.5% NaCl, 0.1% glucose [pH 7.0]). Purified plasmid DNA was centrifuged in linear sucrose gradients (11 ml; 15 to 30%). The gradients were prepared in TES buffer (0.05 M Tris, 0.05 M EDTA, 0.05 M NaCl [pH 8.0]), 0.1 ml of plasmid-containing solution was layered on top of the gradients, and centrifugation was carried out in a Beckman SW41 rotor at 20°C for 120 to 180 min. Sedimentation constants and the molecular weights of individual molecules were calculated according to the method of Hudson et al. (22) and compared with those of reference plasmids.

Agarose gel electrophoresis and restriction endonuclease digestion. Plasmid DNA extraction and agarose gel electrophoresis were performed as described by Meyers et al. (27). In some experiments, the extraction method of Hansen and Olsen (21) or Casse et al. (8) was used to separate any large plasmid from the folded chromosome. DNA was digested with restriction enzymes *Pst*I and *Sma*I according to the instructions of the manufacturer (Boehringer Mannheim Corp., Mannheim, West Germany). Reference plasmids for molecular-weight determinations of plasmids were isolated from *E. coli* V517 (26). A λ *Hin*dIII digest was also used as a molecular-weight standard (29).

Southern transfer, hybridization, and autoradiography. Total DNA was isolated from the strains as described by Cosloy and Oishi (11). *Pst*I-digested samples were subjected to agarose gel electrophoresis. The DNA fragments were denatured and transferred to nitrocellulose filters as described by Southern (35). Plasmid DNA was radiolabeled by nick translation according to the method of Rigby et al. (34). Filters from Southern transfer experiments were hybridized with labeled plasmid DNA (35). Autoradiography of the filters was done with Ilford 100 NIF X-ray films.

RESULTS

Antimicrobial resistance and enzyme studies. The minimal inhibitory concentrations (MICs) of various antimicrobial agents against the epidemic strain HK302 and two variant cultures (variants 1 and 2) which had lost components of their resistance patterns by curing are summarized in Table 1. The wild-type strain was susceptible to tobramycin, netilmicin, and amikacin and highly resistant to the other drugs shown. The strain was also resistant (MIC, ≥32 mg/liter) to cephaloridine, cefamandole, cefoxitin, cefuroxime, and cefoperazone, but susceptible (MIC, ≤8 mg/liter) to ceftazidime, ceftriaxone, cefotaxime, and moxalactam. Carboxypenicillins and ureidopenicillins were inactive against HK302. Variant 1 had lost resistance to kanamycin, neomycin, and ampicillin. Variant 2, a derivative of variant 1 that had undergone further curing treatments, was, in addition, no longer resistant to gentamicin, tetracycline, and sulfamethoxazole. Resistance was difficult to cure from HK302 and variant 1. Treatment of cultures with subinhibitory concentrations of ethidium bromide or storage at 35°C for several weeks yielded only one to two clones per 5,000 colonies screened.

TABLE 1. MICs of various antimicrobial agents against *A. calcoaceticus* strains

Antimicrobial agent	MIC (mg/liter) against:		
	Strain HK302	Variant 1	Variant 2
Gentamicin	>200	>200	0.25
Sisomicin	>200	>200	0.25
Tobramycin	0.25	0.25	0.25
Netilmicin	8	2	1
Amikacin	4	4	2
Kanamycin	>200	2	0.5
Neomycin	>200	4	1
Streptomycin	200	200	200
Chloramphenicol	>200	200	200
Tetracycline	200	200	4
Ampicillin	>200	4	4
Cephalothin	>200	100	100
Sulfamethoxazole	>800	>800	2

Crude enzymatic preparations of the *Acinetobacter* sp. strains that were obtained by the osmotic shock procedure were examined for aminocyclitol-modifying activity. The results of phosphorylation, acetylation, and adenylylation experiments with various aminocyclitol substrates are shown in Table 2. From the profiles, it can be deduced that strain HK302 produced a type 1 3'-phosphotransferase [APH(3')-1], an enzyme widely distributed in gram-negative bacteria (13). In addition, HK302 produced an acetylating enzyme with a profile pattern typical of a type 1 3-*N*-acetyltransferase [AAC(3)-1] (4). Furthermore, HK302 contained the 3"-adenylytransferase [AAD(3")] of gram-negative bacteria (36). The osmotic shock extract also included a penicillinase that was indistinguishable by substrate profile and isoelectric point (5.6) from TEM-2 β-lactamase. No other β-lactamase was observed in the osmotic shock extract. An ultrasound extract, however, contained an additional β-lactamase with strong activity against cephalosporin antibiotics. This cell-bound β-lactamase was not further characterized. HK302 also produced chloramphenicol-inactivating activity (CIA).

TABLE 2. Substrate profiles of aminoglycoside-modifying enzymes isolated from the *A. calcoaceticus* wild-type strain

Substrate	Extent of incorporation of[a]:		
	^{32}P (phosphorylation)	^{14}C (acetylation)	^{3}H (adenylylation)
Neomycin	1,245	63	0
Kanamycin	638	59	43
Lividomycin	733	162	ND[b]
Paromomycin	1,693	58	60
Butirosin	47	59	37
Tobramycin	42	527	ND
Gentamicin	0	8,085	50
Sisomicin	33	9,495	77
Netilmicin	2	747	ND
Amikacin	ND	83	0
Streptomycin	ND	ND	2,177
Spectinomycin	ND	ND	1,667

[a] Counts per minute incorporated and assayed after 2.5 h of incubation at 35°C.
[b] ND, Not done.

A summary of the enzymes of strain HK302 and variants 1 and 2 is given in Table 3. It should be realized that variant 2, although resistant to streptomycin and chloramphenicol (Table 1), did not produce enzymes that modify the two drugs, whereas the other strains did produce such enzymes.

Conjugation studies. Transfer of any resistance from strain HK302 to three *E. coli*, one *P. aeruginosa*, and two *A. calcoaceticus* strains, by both the agar method (37) and the broth method (31), was unsuccessful. Transfer of resistance from HK302 to *Acinetobacter* variant 2 also could not be achieved. It has been shown that nonconjugative plasmids can be transferred in the presence of conjugative plasmids (reviewed in reference 9). It also has been shown that *Acinetobacter* chromosomal genes can be mobilized with the conjugative plasmid RP4 (37). This plasmid specifies resistance to ampicillin, kanamycin, and tetracycline. We transferred plasmid RP4 from *E. coli* K-12 (J53) into the ampicillin- and kanamycin-susceptible variant 1 of HK302 at a frequency of 2×10^{-7} per donor cell. Selection of transconjugants was carried out on agar containing 15 mg of gentamicin and 25 mg of kanamycin per liter. Transfer of plasmid RP4 into the wild type could not be examined, because resistance markers specified by plasmid RP4 were also present in HK302.

Transfer of resistance to gentamicin, streptomycin, sulfamethoxazole, and chloramphenicol together with the plasmid RP4-specified resistance from the plasmid RP4-containing variant 1 to *E. coli* HB101 (*recA*) could be achieved by the agar transfer method (37). Selection was for

TABLE 3. Antibiotic-modifying enzymes in *A. calcoaceticus* strains

Enzyme or activity	Enzymatic activity in:		
	Strain HK302	Variant 1	Variant 2
APH(3')-1	Yes	No	No
AAC(3)-1	Yes	Yes	No
AAD(3")	Yes	Yes	No
TEM-2	Yes	No	No
Cephalosporinase	Yes	Yes	Yes
CIA	Yes	Yes	No

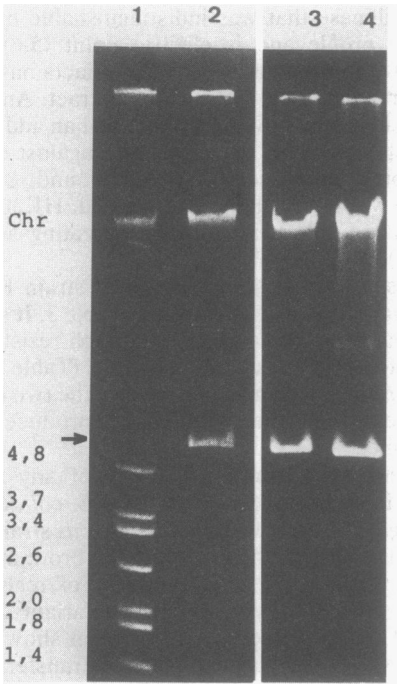

FIG. 1. Agarose gel electrophoresis of the DNA from crude lysates (numbers on the left indicate fragment masses in megadaltons). (1) Reference plasmids; (2) *A. calcoaceticus* HK302 (wild-type strain); (3) *A. calcoaceticus* variant 1; and (4) *A. calcoaceticus* variant 2. Chr, Chromosomal DNA. The arrow indicates a 6-Md cryptic plasmid.

resistance to gentamicin (15 mg/liter) and high-level resistance to streptomycin (2,000 mg/liter). Three transconjugants were examined for the production of antibiotic-modifying enzymes. All three isolates produced AAC(3)-1 and AAD(3″) and were able to inactivate chloramphenicol. A possible transfer of tetracycline resistance from variant 1 to *E. coli* with the help of plasmid RP4 could not be examined, since plasmid RP4 mediates resistance to tetracycline.

The instability of resistance markers in HK302 as well as the mobilization of components of the resistance pattern of variant 1 suggested replicative autonomy of their resistance determinants. We therefore performed experiments to obtain information about the physical autonomy of these markers.

Plasmid DNA studies. *A. calcoaceticus* HK302, as well as antibiotic-susceptible variant 1 and variant 2, each harbored a 6-megadalton (Md) plasmid (Fig. 1). The faint bands above the covalently closed circular form of the 6-Md plasmid probably represent the open circular form of this plasmid, since the open circular forms of small plasmids migrate a shorter distance than the covalently closed circular forms in agarose gel electrophoresis (20). The 6-Md plasmid, thus, has nothing to do with the unstable resistances of HK302. To separate from the folded chromosome any large plasmid that might be present, the plasmid DNA isolation procedures of Hansen and Olsen (21) and Casse et al. (8) were used. With the exception of the 6-Md plasmid, no other plasmid DNA was ever observed.

Examination of the plasmid content of a transconjugant obtained by mating plasmid RP4-containing variant 1 with *E. coli* HB101 (*recA*) (see above) revealed the presence of a 51.7-Md R plasmid (pFK20). Thus, plasmid pFK20 mediates the resistance to ampicillin, kanamycin, and tetracycline that originates from plasmid RP4 and the resistance to chloramphenicol (CIA), gentamicin [AAC(3)-1], streptomycin [AAD(3″)], sulfamethoxazole, and possibly also tetracycline that originates from variant 1. Since the vector plasmid has a molecular mass of approximately 36 Md (15), the DNA sequence which presumably was transposed from the *Acinetobacter* DNA to plasmid RP4 was about 16 Md (Fig. 2).

To obtain additional information about the transposon nature of this sequence, resistance to gentamicin (15 mg/liter) was transferred from the *E. coli* transconjugant into *Acinetobacter* variant 2 (Nal^r) by broth mating (31). The transfer frequency was 2×10^{-7} per donor cell. The resistance phenotypes of 10 transconjugant clones were examined by a single-disk diffusion method. The phenotypes were indistinguishable from the resistance phenotype of variant 1. No satellite DNA other than the 6-Md plasmid was detected by gel electrophoresis in these transconjugants.

We also did not observe any plasmid DNA in 10 kanamycin- and ampicillin-susceptible variants of *E. coli* HB101 [*recA*(pFK20)]. These variants, however, were still resistant to gentamicin, streptomycin, chloramphenicol, sulfamethoxazole, and tetracycline. They arose spontaneously in overnight broth cultures at a frequency of 75%.

Thus, all results indicated that the markers for resistance to gentamicin [AAC(3)-1], streptomycin [AAD(3″)], chloramphenicol (CIA), sulfamethoxazole, and tetracycline in the *Acinetobacter* variant 1 as well as in plasmid pFK20 were part of a 16-Md transposon.

Characterization of DNA by restriction endonuclease digestion. Cleavage of plasmid RP4 with *Pst*I resulted in four fragments of approximately 16.8, 14.5, 4, and 1.9 Md (Fig. 3, track 4). This result is in accord with data given by DePicker et al. (15). Due to their small size, two 0.5-Md fragments described by those authors could not be visualized with the electrophoresis procedure

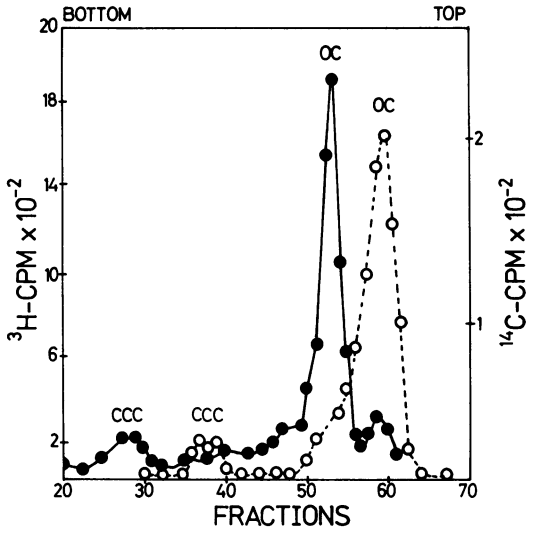

FIG. 2. Sedimentation of purified ^{3}H-labeled plasmid RP4 DNA (○) and ^{14}C-labeled recombinant plasmid pFK20 DNA (●) on a 15 to 30% neutral sucrose gradient. Density increases from right to left. CCC, Covalently closed circular DNA; OC, open circular DNA. S_{oc} (Svedberg) of RP4, 43; S_{oc} (Svedberg) of pFK20, 50.

we used. Digestion of recombinant plasmid pFK20 produced five additional DNA fragments of 8, 4, 1.8, 1.6, and 1 Md (Fig. 3, track 3). The 4-Md piece very probably was a doublet. The sum of the molecular masses of the additional DNA fragments of plasmid pFK20 was 16.4 and thus was in good accord with the increase in mass of plasmid RP4 after insertion of the *Acinetobacter* DNA.

Digestion of plasmid RP4 with *Sma*I resulted in four fragments of 15, 10.5, 9.3, and 4.4 Md (Fig. 3, track 5). Cleavage of plasmid pFK20 gave similarly sized fragments (Fig. 3, track 2). The 15-Md band was a doublet. Unfortunately, in the example presented, the presence of two 15-Md pieces cannot be recognized easily because of nonspecific cleavage of DNA by endonucleases. Digestion of this band with *Pst*I, however, showed that the band was composed of two DNA pieces with different *Pst*I cut sites (data not shown). One of these fragments represents the transposed *Acinetobacter* DNA.

Based on the digestion results, the *Acinetobacter* sequence apparently has *Pst*I- and *Sma*I-specific sites near both ends, and insertion of *Acinetobacter* DNA into plasmid RP4 occurs near the *Pst*I and *Sma*I sites of the plasmid. A physical map of plasmid RP4 shows both endonucleases have a cut site around position 22.5 (15), suggesting, therefore, that the *Acinetobacter* transposon was inserted near this position (14).

Hybridization studies. Total DNA from variant 1 was isolated and digested with *Pst*I. Figure 4A, track 1, shows the digestion pattern obtained, track 2 shows the DNA fragments of the total DNA from variant 2, and track 3 shows the pattern obtained after digestion of plasmid pFK20. DNA fragments were transferred to nitrocellulose filters, subsequently hybridized with ^{32}P-labeled DNA of plasmid pFK20, and autoradiographed with X-ray film. Figure 4B, track 1, shows that the transposon-specific fragments of the recombinant plasmid pFK20 were present in the total DNA from *Acinetobacter* variant 1. However, the 8- and 1.8-Md *Pst*I fragments of the chromosomal digest (Fig. 4B, track 1) increased slightly in mass. These fragments probably represent the two outermost fragments of the transposable element, now flanked by a different host sequence. No homology between the DNA of plasmid pFK20 and the DNA from variant 2 was observed (Fig. 4B, track 2).

DISCUSSION

Acinetobacter calcoaceticus subsp. *anitratus* caused epidemic nosocomial infections in patients hospitalized at the University of Zurich hospital (16). The isolates cultured during the epidemic were found to be resistant to many antimicrobial agents. Examination of the mecha-

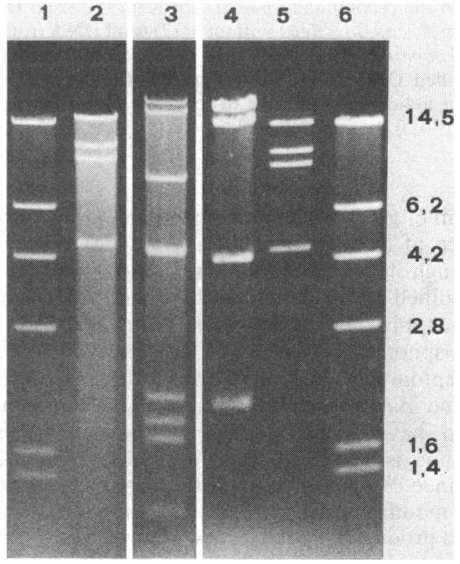

FIG. 3. Agarose gel electrophoresis of the *Pst*I and *Sma*I digest fragments of plasmids RP4 and pFK20. (1 and 6 *Hin*dIII-digested λ DNA (numbers on the right indicate fragment masses in megadaltons); (2) *Sma*I-digested plasmid pFK20; (3) *Pst*I-digested plasmid pFK20; (4) *Pst*I-digested plasmid RP4; and (5) *Sma*I-digested plasmid RP4.

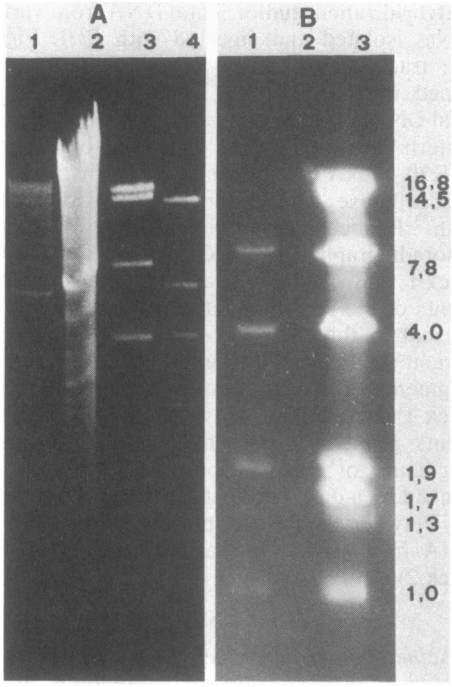

FIG. 4. (A) Agarose gel electrophoresis of *Pst*I digest fragments of chromosomal and plasmid DNA. (1) Total DNA from *A. calcoaceticus* variant 1; (2) total DNA from *A. calcoaceticus* variant 2; (3) *Pst*I-digested DNA from plasmid pFK20; and (4) *Hin*dIII-digested λ DNA. (B) Autoradiogram after hybridization of *Pst*I digest fragments with ^{32}P-labeled DNA from the recombinant plasmid pFK20. (1) Total DNA from *A. calcoaceticus* variant 1; (2) total DNA from *A. calcoaceticus* variant 2 (no hybridization); and (3) purified DNA from plasmid pFK20 (numbers on the right indicate the masses of the *Pst*I digest fragments of pFK20 in megadaltons).

nism of gentamicin resistance revealed the presence of the acetylating enzyme AAC(3)-1. Although tobramycin and netilmicin were also modified by this enzyme, the strains tested were susceptible to these drugs. Furthermore, the phosphotransferase APH(3′)-1, as well as the streptomycin- and spectinomycin-modifying enzyme AAD(3″) were present. These enzymes also have been found in *Acinetobacter* sp. strains isolated in the United States (28) and France (3). In addition to the three aminocyclitol-modifying enzymes, the epidemic strains also produced a periplasmic penicillinase (TEM-2), which renders cells resistant to ampicillin, carboxypenicillins, and ureidopenicillins, and a cell-bound cephalosporinase, which contributes to resistance against a variety of cephalosporin antibiotics.

We tried to reconstruct the evolution of multiple antibiotic resistance in strain HK302. The easiest explanation was infection of susceptible *Acinetobacter* sp. strains, such as the HK302 variant 2, with a conjugative R plasmid. The resistance pattern of the epidemic strain is frequently observed among members of the *Enterobacteriaceae* family that constitute hospital flora. However, no transfer of resistance from *Acinetobacter* sp. strains to various recipients could be achieved, and no evidence of plasmid-mediated resistance of the unstable markers of HK302 was obtained in plasmid DNA isolation studies. Resistance to various drugs, however, could be transferred with the help of plasmid RP4. DNA analysis of transconjugants showed that mobilization by plasmid RP4 was accompanied by a 16-Md increase in the mass of the plasmid. We strongly suggest that the 16-Md DNA sequence was transposed from the *Acinetobacter* chromosome to plasmid RP4. The formation of a cointegrate (24) between an *Acinetobacter* R plasmid and plasmid RP4 is improbable, because no plasmid DNA other than a 6-Md cryptic plasmid was detected. Furthermore, transposition of the transposon resistance markers from plasmid pFK20 into the chromosome of *Acinetobacter* variant 2 and into the chromosome of a *rec*-deficient *E. coli* was demonstrated. Comparative analysis of *Pst*I and *Sma*I digests of plasmids RP4 and pFK20 suggested that the insertion of the *Acinetobacter* transposon had occurred at position 22.5 on the physical map of plasmid RP4. The transposon mediated resistance to gentamicin [AAC(3)-1], streptomycin [AAD(3″)], chloramphenicol (CIA), sulfamethoxazole, and probably also tetracycline. It is not known whether resistance markers for ampicillin (TEM-2) and kanamycin [APH(3′)-1] in HK302 originally belonged to this cluster of resistance determinants also.

For the evolution of strain HK302, we hypothesize that an *Acinetobacter* sp. strain already intrinsically resistant to chloramphenicol and streptomycin and resistant to various cephalosporins due to a cephalosporinase became infected with a conjugative R plasmid. This plasmid mediated resistance to ampicillin and kanamycin, as well as to chloramphenicol, gentamicin, streptomycin, sulfamethoxazole, and tetracycline. The plasmid could not be maintained stably in the *Acinetobacter* host. However, transposition and stable integration of the DNA sequence encoding antibiotic resistance into the *Acinetobacter* chromosome occurred. Such events can have serious epidemiological and therapeutic implications.

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